

Journal Homepage: www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/17359

DOI URL: <http://dx.doi.org/10.21474/IJAR01/17359>

RESEARCH ARTICLE

EFFECTS OF RELEASES OF THE EGG PARASITOID *GRYON FULVIVENTRE* CRAWFORD (HYMENOPTERA: SCELIONIDAE) ON *CLAVIGRALLA TOMENTOSICOLLIS* STÅL. (HEMIPTERA: COREIDAE), A COWPEA POD-SUCKING BUG, IN A CONTROLLED ENVIRONMENT

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Manuscript Info

Manuscript History

Received: 05 June 2023

Final Accepted: 09 July 2023

Published: August 2023

Key words:-

Egg Parasitoids, Insect Pest, Artificial Infestation, Parasitism Rate, Frequency

Abstract

Clavigralla tomentosicollis Stål. (Hemiptera: Coreidae), major cowpea pod-sucking bug, causes significant economic losses. A promising alternative to chemical control of this pest is the use of the egg parasitoid *G. fulviventre*. Given that no release strategy for *G. fulviventre* has yet been tested, it would seem wise, before considering large-scale use of this parasitoid, to determine an optimum release frequency. The aim of this study is to find an optimal frequency of parasitoid release. Artificial infestations were carried out in a semi-controlled environment at the Center for Environmental, Agricultural Research and Training of Kamboinsé (Burkina Faso). A single release of the pest was carried out with five release frequencies of the parasitoid. The results showed that the rate of parasitism was higher ($64.57 \pm 4.76\%$) in successive releases of the parasitoid spaced one week apart and lower ($42.72 \pm 4.99\%$) in the single release. Pods were almost completely damaged without parasitoid release ($91.83 \pm 1.74\%$) and less damaged with successive releases ($24.58 \pm 1.59\%$). Parasitism rates did not differ significantly between two, three or four successive releases. This study is a first on the release of *G. fulviventre* in Burkina Faso and the results are of great importance for the implementation of an integrated management strategy of *C. tomentosicollis*.

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Introduction:-

Cowpea, *Vigna unguiculata* L. (Walp) (Fabales: Fabaceae), is a legume with high potential that is now considered as a commodity chain that can contribute to food security and poverty reduction. The annual demand for cowpea is increasing by 3.87% in Burkina Faso and 3.60% in Nigeria (Sanginga and Bergvinson, 2015). This growing demand for cowpea can be explained by the many uses and benefits that cowpea provides. Indeed, the grain and leaves are used in human nutrition. They are an important source of protein, minerals and vitamins. The leaves are eaten as a sauce or mixed with cereals. They contain 34.91% of carbohydrates (Boukar et al., 2011; Enyiukwu et al., 2018). Rich in polyphenols and antioxidants, cowpea consumption is highly beneficial for human health, especially for children. The leaves and stems are an excellent fodder for livestock, which can improve milk production in cows

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(Pousga et al., 2019). Agronomically, it enriches the soil with nitrogen through the symbiotic bacteria in its roots, which benefits other crops. The sale of cowpea and its processed products is a source of income for households.

In Burkina Faso, cowpea yields remain low despite the use of improved varieties (Ishikawa et al., 2013). The low productivity of cowpea is explained by several biotic and abiotic factors. Insect pests are the major constraint responsible for the decline in cowpea yield (Singh et al., 1992).

Among these insects is *C. tomentosicollis*, the main species of cowpea pod-sucking bug responsible for economic damage to this crop in Burkina Faso and West Africa (Rabe et al., 2017; Bello et al., 2018; Baoua et al., 2021). Given the recurrence of outbreaks and the inadequacy of current control methods, which are mainly based on synthetic insecticides, biological control of *C. tomentosicollis* is being considered. This method is part of the Integrated Pest Management (IPM) approach that has been explored in cowpea crops for two decades with the implementation of cultural practices, the use of resistant varieties and biopesticides (Dabiré, 2001; Koono et al., 2002; Ba et al., 2009). However, these methods have limitations as they often require the additional use of synthetic insecticides to optimise yields (Sharma, 1998).

To find a sustainable and ecologically acceptable solution for the management of *C. tomentosicollis* populations, research is currently focusing on biological control, including the use of parasitoids as a promising component of IPM. For example, the oophagous parasitoid *G. fulviventre* (Hymenoptera: Scelionidae) offers hope due to its remarkable natural occurrence in agroecosystems (Asante et al., 2000; Dabiré, 2001) and its ability to detect the presence of the pest using olfactory cues (Sanou et al., 2019). Therefore, the oophagous parasitoid *G. fulviventre* could be used for biological control through augmentative releases. However, the success of augmentative releases of a given natural enemy depends on several parameters such as time, frequency and release rate of that natural enemy (Smith, 1996).

In addition, in the case of oophagous parasitoids, synchronisation between the time of parasitoid release and the onset of pest oviposition is required for greater efficiency (Smith, 1994). In the case of *G. fulviventre*, as no release strategy has yet been tested, it seems prudent, before considering the large-scale use of this parasitoid, to determine an optimal release frequency that could reduce the pest population level and, at the same time, significantly reduce its damage.

The present study was carried out under semi-controlled conditions in a greenhouse with plants artificially infested by *C. tomentosicollis*. The aim was to evaluate different release scenarios in order to identify an optimal release scenario and timing of the parasitoid *G. fulviventre* for the control of cowpea pod bug infestation.

Material And Methods:-

Plant material

The new improved variety KOMCALLE appreciated by the farmers was used in the trials. Its semi maturity cycle is sixty days after sowing (60 DAS). It is a variety grown in regions with rainfall ranging between 400 and 800 mm (Ouédraogo et al., 2011).

Animal material

The insects used for the artificial infestation were obtained from a mass rearing at the Entomology Laboratory of the Institute for Environmental and Agricultural Research (INERA) at Kamboinsé (12°28'N 32°1'W), Ouagadougou, Burkina Faso (West Africa). Newly emerged adults of the pest *C. tomentosicollis* and the parasitoid *G. fulviventre* were used.

Technical material

The 1 m² covers were made of fine-meshed fabric that did not allow the parasitoids to escape. Each cover has two openings through which the hand can be inserted to carry out the releases and observations.

Composition of treatments

Cowpea was sown in pots at a rate of two plants per pot. Four pots of cowpea were planted under each cover. This plot is considered to be an elementary plot. The cowpea plants received an early treatment against thrips with deltamethrin on the 30th day of the week before covering.

The releases started at the beginning of pod formation, 42 days after sowing. They consisted of the release of a single pair of *C. tomentosicollis*. The different release scenarios of *G. fulviventris* were used in four different treatments: Treatment T0, control without parasitoid; Treatment T1, a single release at parasitoids on 42 DAS; Treatment T2, two releases of parasitoids at 42 and 49 DAS; Treatment T3, three releases of parasitoids at 42; 49; 56 DAS; Treatment T4, four releases of parasitoids at 42; 49; 56; 63 DAS and Treatment T5, two releases of parasitoids at 42 and 56 DAS.

Method of release

Considering the pre-oviposition period found by Abdourahamane-Harouna et al. (2018), the five (5) day old *C. tomentosicollis* adults were isolated in pairs in the petri dishes. They were then introduced into the cages. For the parasitoid, three pairs of 24 hours old *G. fulviventris* were isolated in the 3.3 cm x 1.2 cm boxes. Once the boxes were placed in the cages, they were opened to allow the individuals to escape.

Parameters studied

Observations began one week after the first release and continued until the cowpea maturity. Observations consisted of identifying two clusters of *C. tomentosicollis* eggs and counting the total number of eggs and the number of parasitised eggs, recognisable by the black colour. These two parameters were used to calculate the parasitism rate for each treatment. At cowpea harvest, the total number of pods and the number of deformed pods were counted to estimate damage.

Data analyses

Data were exported from Excel to RAWGraphs 2.0 beta (Mauri et al., 2017) for graph construction. Analysis of variance (ANOVA) was performed using SAS version 9.1 software (SAS 2003). Where differences between treatments were significant, means were separated using the Student-Newman-Keuls test at the 5% probability level.

Results:-

Evolution of *C. tomentosicollis* oviposition in the treatments

The *C. tomentosicollis* females released into the cages laid eggs continuously until the cowpea reached maturity (Fig. 1). However, oviposition was non-linear in all treatments except the T0 control. Regardless of the treatment, the mean number of eggs laid at 49 and 63 DAS was not significantly different (49 DAS: $F_{5;18} = 1.92$, $P = 0.14$ 63 DAS: $F_{5;18} = 1.01$, $P = 0.43$). On the other hand, there was a significant difference in the number of eggs laid at 56 and 70 days according to treatment (56 DAS: $F_{5;18} = 2.89$; $P = 0.04$; 70 DAS: $F_{5;18} = 8.19$; $P = 0.0004$) (Fig. 1).

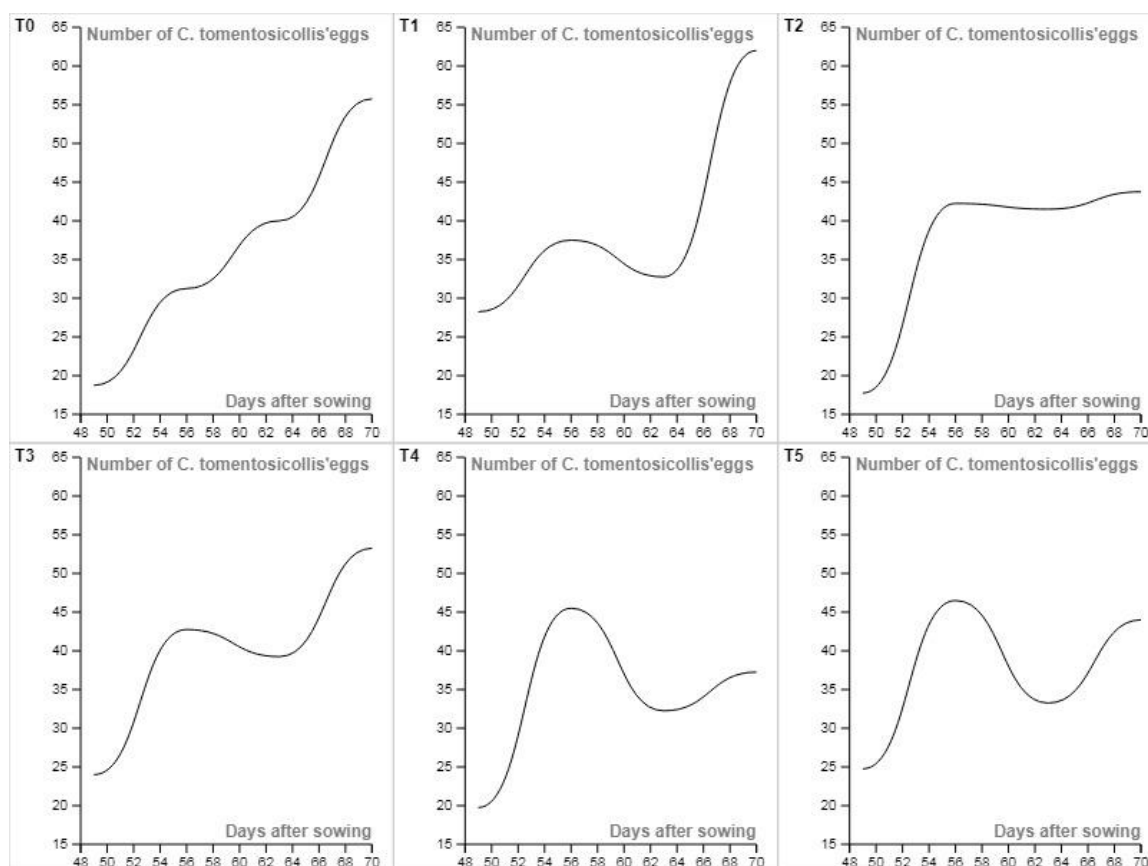


Fig. 1:- Evolution of the number of eggs laid by *C. tomentosicollis*.

Evolution of *G. fulviventre* parasitism in the different treatments

The rate of parasitism was not significantly different between treatments at 49 and day 63 DAS (49 DAS: $F_{4; 15} = 0.27$, $P = 0.89$; 63 DAS: $F_{4; 15} = 2.35$, $P = 0.10$) (Fig. 2). The rate of parasitism differed significantly between 56 and 70 DAS (56 DAS $F_{4; 15} = 16.71$; $P = 0.0001$; 70 DAS: $F_{4; 15} = 54.92$; $P = 0.0001$) (Fig. 2). At 56 days, parasitism was not significantly different between treatments T2, T3 and T4, which had the highest parasitism rates. However, parasitism in these three treatments was significantly different from parasitism in T1 and T5 (Fig. 2).

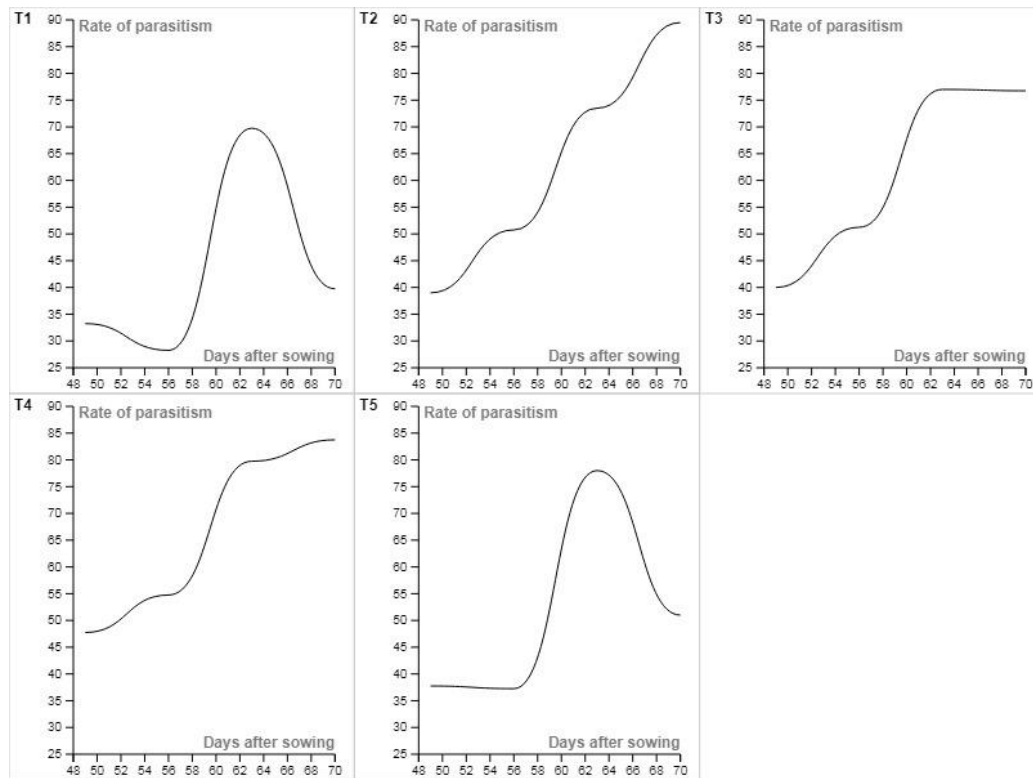


Fig. 2:- Evolution of the rate of parasitism of *G. fulviventris* on the eggs of *C. tomentosicollis*.

Effect of releases of the parasitoid *G. fulviventris* on *C. tomentosicollis* eggs

A significant difference was observed in the mean number of *C. tomentosicollis* eggs remaining after parasitism by *G. fulviventris* during the observation dates, except for the first observation date, which was the 49 DAS ($F_{5; 18}=1.98$, $P=0.13$) (Fig. 3). At 56 and 70 DAS, the number of eggs did not differ significantly between treatments T2, T3 and T4. However, a significant difference in the number of eggs was observed between these three treatments and treatments T0, T1 and T5 (56 DAS: $F_{5; 18}=4.38$, $P=0.008$; 70 DAS: $F_{5; 18}=56.05$, $P=0.001$) (Fig. 3). For the 63 DAS, the number of eggs in treatments T1, T2, T3, T4, T5 was significantly different from that in treatment T0 ($F_{5; 18}=33.59$, $P=0.001$).

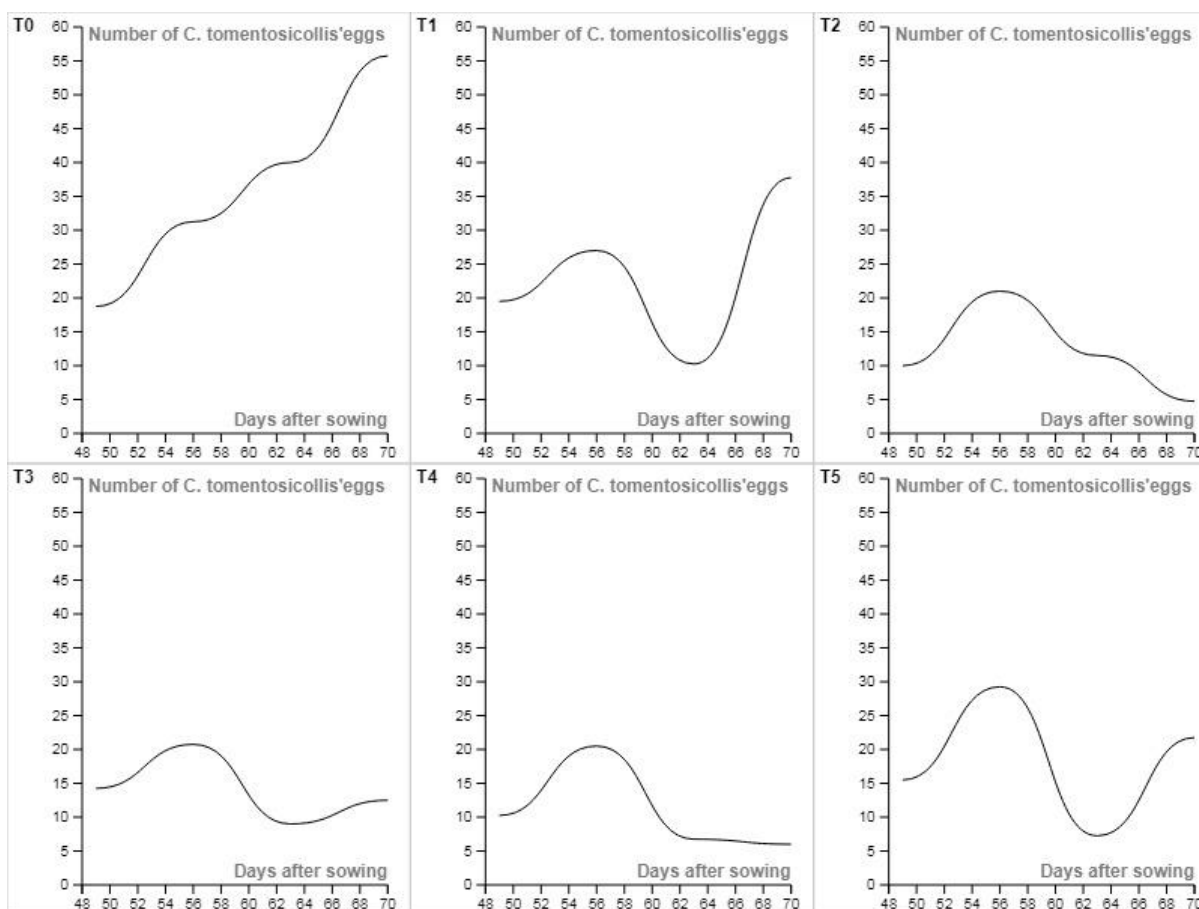


Fig. 3:- Evolution of *C. tomentosicollis* eggs number after parasitism.

Global parasitism rate in the different treatments

The number of eggs laid by *C. tomentosicollis* females was not statistically different between treatments. However, the different ways of releasing the parasitoid *G. fulviventris* had a significant effect ($F_{5,90}=29.50$; $p = 0.001$) on the overall parasitism rate (Table 1). In fact, treatments T2, T3 and T4 had the highest parasitism rates, but they were not statistically different from each other.

Table 1:- Average number of *C. tomentosicollis* eggs, number of parasitized eggs and parasitism rate.

Treatments	Average number of eggs laid by <i>C. tomentosicollis</i> (\pm SD)	Parasitism rate of <i>G. fulviventris</i> ($\% \pm$ SD)
T0	36.44 \pm 3.74	-
T1	40.13 \pm 3.82	42.72 \pm 4.99 a
T2	36.31 \pm 3.59	63.11 \pm 6.19 b
T3	39.81 \pm 3.05	61.29 \pm 4.43 b
T4	34.25 \pm 3.2	64.57 \pm 4.76 b
T5	38.00 \pm 3.38	48.26 \pm 4.33 a
	$F_{5, 87} = 38.7$	$F_{5, 87} = 57.39$
	$P = 0.45$	$P = 0.001$

Means within a column followed by the same alphabetical letters do not differ significantly at the level of 5 %

Pod production and pod damage by *C. tomentosicollis*

Pod production in treatments receiving at least one parasitoid release was not statistically different (Table 2). However, pod production was significantly higher in the treatments that received releases than in the control, which received no parasitoid release ($F_{5,18} = 5.66$; $p = 0.002$). Pod damage by the parasitoids was much higher in the

control, with an average percentage of damaged pods of 91.83% (Table 2). The lowest damage (24%) was observed in treatments with at least two consecutive parasitoid releases.

Table 2:- Pod production and pod damage in the treatments.

Treatment	Total number of pods (\pm SD)	Percentage of damaged pods (\pm SD)
T0	29 \pm 5.4b	91.83 \pm 1.74 a
T1	50.75 \pm 4.78a	63.78 \pm 2.49 b
T2	58.5 \pm 3.66a	24.75 \pm 0.49 d
T3	55.75 \pm 2.56 a	24.58 \pm 1.59 d
T4	64.25 \pm 4.55 a	25.98 \pm 0.70 d
T5	41.75 \pm 8.98 ba	42.07 \pm 3.1 c
	F _{5; 18} = 5.66 P = 0.0026	F _{5; 18} = 202.81 P = 0.001

Means within a column followed by the same alphabetical letters do not differ significantly at the level of 5 %

Discussion:-

The results showed that *C. tomentosicollis* females were continuously laying eggs throughout the observation period. This means that the pest was in an environment similar to its natural habitat, as the establishment of an insect in a given habitat depends on the optimal biotic and abiotic conditions for its survival (Hanski, 2005; Kawecki, 2008). Indeed, the infestation period at 42 DAS, corresponding to pod formation, was favourable for egg deposition and larval development of *C. tomentosicollis*, as females tend to lay their eggs on host plants or habitats that are most suitable for the development of their offspring (Videla et al., 2012; Jones et al., 2019). Similarly, oviposition of *C. tomentosicollis* has been observed in the field at the same time of the cowpea cycle (Dabiré, 2001). The oviposition pattern observed is due to the biology of the pest, *C. tomentosicollis*, which lays eggs for about 10 weeks with a peak in female oviposition between the second and fifth week of life (Dabiré, 2001).

Endogenous natural enemies are present in field crops at variable densities depending on biotic and abiotic factors. Their natural control does not always keep pest populations below economic thresholds. It can therefore be useful to increase the numbers of these natural enemies through supplementary releases: this is the augmentative biological control approach. For egg parasitoids, these releases should be made before the onset of host oviposition (Sithanantham et al., 2001). Furthermore, the success of biological control depends on the dispersal ability of the parasitoid (Mills and Heimpel, 2018; Baoua et al., 2018). In our study, the releases of *G. fulviventris* were conducted in cages and reduced environments, but the results suggest that they established there by giving new generations. Previous results on the dispersal of another species of the same genus, *Gryon gallardoi* (Hymenoptera: Scelionidae), have shown that females have a dispersal capacity of at least seven meters (Canto-Silva et al., 2006).

Analysis of the parasitism of *C. tomentosicollis* eggs in the release cages (T1-T5) showed that it increased with the number of parasitoid releases. Treatments T1 and T5 (one release and two releases at two weeks apart respectively) did not show a continuous increase in parasitism rate over time. This is due to the biology of the insect, where the majority of eggs are laid in the first week of life, as is the case in the genus *Gryon* (Martel, 2019). The second release of T5 at 56 DAS coincides with the emergence of the first generation of the first release, which explains the peak in parasitism observed at 63 DAS in these two treatments.

Treatments T2, T3 and T4, with at least two continuous releases one week apart, show a parasitism rate that progressively increases over time. This increase is due to the effect of subsequent releases from the 49 DAS, one week after the first release. Moreover, there was no difference between these three treatments, two, three and four regular releases. Our results are similar to some previous observations. For example, regular releases (five in total) of *Gryon philippinense* Ashmead (Hymenoptera: Scelionidae) at five-day intervals against *Acanthocoris sordidus* (Hemiptera: Coreidae), a pest of eggplant and pepper, achieved a parasitism rate of 98.8% after four months of experimentation (Dasilao and Arakawa, 2005).

Similar results were also obtained with the release of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) on *Tuta absoluta* (Lepidoptera: Gelechiidae) eggs (Cherif et al., 2019) and the release of *Trichogramma chilonis* Ishii against *Chilo sacchariphagus* Indicus (Lepidoptera: Crambidae) (Geetha et al., 2010).

The frequency of release of a natural enemy for effective biological control depends on the species. In the genus *Trichogramma*, releases are effective from a single release in some species and from more than ten in others (Smith, 1996).

In contrast, some authors suggest that multiple releases are not necessary, but rather the number of parasitoids released once (Li et al., 2006). Thus, in our study, parasitoid releases can be limited to two consecutive releases, which gave the same results as other release scenarios. Furthermore, parasitism increases with the number of *G. fulviventre*, resulting in a high parasitism rate at the end of the cowpea cycle, as in the case of natural parasitism (Asante et al., 2000; Dabiré, 2001).

Pod damage by insects also varied with treatment. Indeed, cowpea pods in control cages (no parasitoid releases and no insecticide treatment) were almost completely damaged (>90%), as suggested by Dreyer (1994). Consistent with parasitism rates, damage was lower in treatments with at least two consecutive releases (T2-T4). These releases, by reducing the number of bugs, also reduce their impact on the pods, hence the low damage.

Although the damage was reduced with regular releases, it remained high due to the low parasitism rate at 49 and 56 DAS, which did not keep the number of *C. tomentosicollis* below the action threshold of four insects per ten cowpea plants (Jackai et al., 1989). On the other hand, with regard to the parasitism rate, it can be said that the releases carried out in treatments T2, T3 and T4 were successful, since, according to Smith (1996), a release of parasitoid is effective if at least 60-80% parasitism and a 77-92% reduction in damage are obtained.

Conclusion:-

For the first time in the context of Burkina Faso, *G. fulviventre* release trials were conducted in a semi-controlled environment to control *C. tomentosicollis* populations from artificial infestations. The study contributes to the determination of an optimal release scenario of *G. fulviventre* for the biological control of *C. tomentosicollis*. Although the study was carried out in a greenhouse under semi-controlled conditions with an artificial infestation, the results obtained indicate the possibility of controlling *C. tomentosicollis* populations by targeted releases. In fact, the best results in terms of parasitism rate and pod damage were obtained in treatments with at least two regular and consecutive parasitoid releases, one week apart. These results provide a reference for carrying out trials in a real environment, while optimising the number of *G. fulviventre* parasitoids to be released.

Acknowledgements:-

We thank the Institut of Environment and Agricultural Research (INERA) of Kamboinsé, Burkina Faso, for the facilities offered during the work. We also thank Ouédraogo Issa for his contribution to set up the trial.

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